

REPRODUCTIVE AND DEVELOPMENTAL DIFFERENTIATION WITHIN SECTION PRIMIGENIA OF THE GENUS CLARKIA

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ABSTRACT.—Section *Primigenia* consists of three subsections. Five diploids are contained in two of these and the third consists of a single allotetraploid. Diploids of different subsections are separated genetically by numerous and extremely well developed isolating mechanisms. Those within the same subsection are not so well isolated from each other. Various lines of evidence indicate that the allotetraploid has combined features and genomes of the two diploid subsections. Such well developed isolating mechanisms have apparently set the stage for natural polyploidy which seems to knit the entire genus.

The genus *Clarkia* is a close relative of the well known *Oenothera* and this is indeed evident in their chromosomal systems. *Clarkia* has 36 species, 24 of which are diploids and the rest are allotetraploids. Most of the species are indigenous to California. The species are annual and occur in separate colonies that vary in size (Lewis and Lewis, 1955). The gene pools of the diploid species are without exception separated by strong barriers to gene exchange. Some species cannot be crossed to one another; interspecific hybrids that can be produced are usually sterile. Such sterility is invariably associated with extensive structural rearrangement of the chromosomes, with or without a change in basic number (Lewis, 1953a).

In the course of their evolution, the diploid species taken as a whole, have become adapted to a diversity of habitats within the plant com-

munities that represent modern derivatives of the Madro-Tertiary Geoflora (Lewis, 1953b). There is a strong correlation between habitat preference and phylogenetic sequence. The derivative species occupy more xeric and presumably more recent habitats than their progenitors (Lewis, 1953b). The pattern of relationships within the genus is one of diverse lineages, and the species have sometimes been referred to separate genera. Natural polyploidy serves to knit the entire group together (Lewis and Lewis, 1955).

The genus is subdivided into 10 sections, of which *Primigenis* seems to be the oldest (Lewis and Lewis,

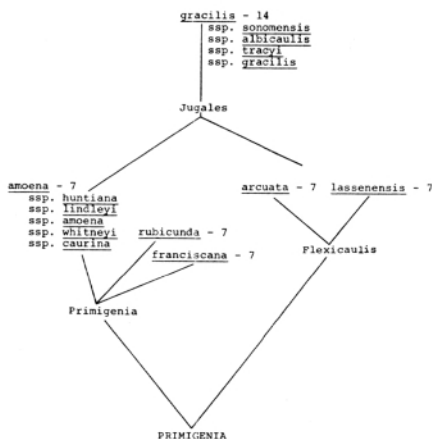


FIGURE 1. Inferred phylogenetic relationships in section *Primigenia*.

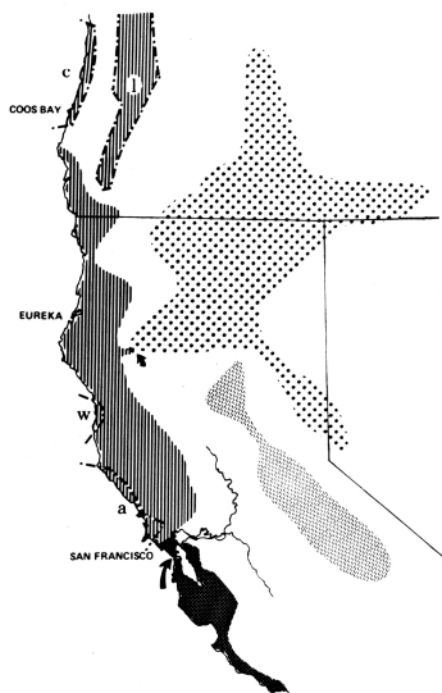
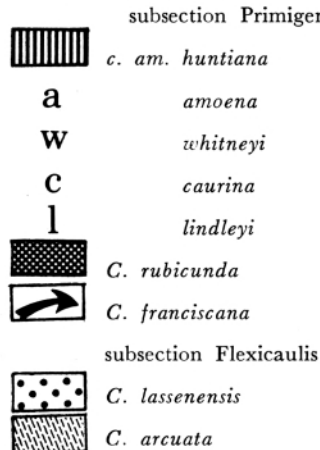


FIGURE 2. Distribution in northern California and adjacent parts of Nevada and Oregon of the five diploids in the section Primigenia. The third subsection, Jugales, is composed of a single polytypic species whose four subspecies overlap the distribution of the five diploids (see, Lewis and Lewis, 1955; Abdel-Hameed, 1967).



1955). Section Primigenia includes 6 species grouped into three different subsections (Figure 1). Primigenia and Flexicaulis subsections contain 5 diploids some of which are considered to be the most primitive members of the genus (Lewis and Lewis, 1955). The third subsection, i.e. Jugales, consists of an allotetraploid that combines morphological and ecological features of the other two subsections; its range overlaps that of the other five species (Figure 2). The purpose of the present study is to examine the extent of reproductive and developmental differentiation between species of these three subsections.

MATERIALS AND METHODS

Bulk collections of seeds were made from wild populations by combining the seeds from a number of capsules collected at random, one from each plant.

Plants were grown from these seeds in 5 inch plastic pots containing a uniform soil mix consisting of 2 parts sandy loam :1 part peat moss. They were occasionally fertilized during the growing season.

The pollination procedure varied according to the breeding habit. Self-pollination did not require emasculation; cross-pollination, however, required emasculation of the female parent. Emasculation was practiced when the stigma lobes were still closed and the anthers had not yet shed their pollen. Pollinations were done when the stigma became receptive. The flowers were protected from unwanted pollen by covering them with cellophane peanut bags. Bags were removed after fertilization had occurred.

TABLE 1.—Intra- and Interspecific Hybrids Between Representative Taxa in Section *Primigenia*.

	Number of Crosses	Number of Plants	Average Percentage of Stainable Pollen ± Standard Error
I. Intra-subspecific Hybrids:			
A. ♀ <i>C. g. sonomensis</i> x ♂ <i>C. g. sonomensis</i>	11	23	77.3 ± 8.04
B. ♀ <i>C. g. tracyi</i> x ♂ <i>C. g. tracyi</i>	3	9	86.3 ± 9.78
C. ♀ <i>C. g. gracilis</i> x ♂ <i>C. g. gracilis</i>	2	2	88.2 ± 8.47
Subtotal.....	16	34	83.9 ± 8.76
II. Inter-subspecific Hybrids:			
A. ♀ <i>C. g. tracyi</i> x ♂ <i>C. g. albicaulis</i>	4	10	77.0 ± 9.23
B. ♀ <i>C. g. sonomensis</i> x ♂ <i>C. g. albicaulis</i>	2	4	33.3 ± 4.68
C. ♀ <i>C. g. sonomensis</i> x ♂ <i>C. g. tracyi</i>	3	9	75.0 ± 7.63
D. ♀ <i>C. g. tracyi</i> x ♂ <i>C. g. sonomensis</i>	3	7	62.2 ± 8.31
E. ♀ <i>C. g. albicaulis</i> x ♂ <i>C. g. sonomensis</i>	2	2	33.7 ± 5.64
F. ♀ <i>C. g. sonomensis</i> x ♂ <i>C. g. gracilis</i>	8	20	70.0 ± 9.26
G. ♀ <i>C. g. gracilis</i> x ♂ <i>C. g. sonomensis</i>	5	5	65.8 ± 6.73
H. ♀ <i>C. g. gracilis</i> x ♂ <i>C. g. tracyi</i>	2	6	62.3 ± 8.34
I. ♀ <i>C. g. tracyi</i> x ♂ (<i>sonomensis</i> x <i>gracilis</i>)..	1	3	75.0 ± 7.67
Subtotal.....	30	66	62.1 ± 7.50
III. Triploids with <i>C. amoena huntiana</i> :			
A. ♀ <i>C. g. sonomensis</i> x ♂ <i>C. am. huntiana</i> ...	32	98	9.5 ± 0.62
B. ♀ <i>C. am. huntiana</i> x ♂ <i>C. g. albicaulis</i>	1	1	2.8 ± 0.31
C. ♀ <i>C. g. tracyi</i> x ♂ <i>C. am. huntiana</i>	19	57	5.0 ± 0.47
D. ♀ <i>C. am. huntiana</i> x ♂ <i>C. g. gracilis</i>	10	30	4.7 ± 0.86
Subtotal.....	62	176	5.5 ± 0.57
IV. Triploids with <i>C. lasseensis</i> :			
A. ♀ <i>C. g. sonomensis</i> x ♂ <i>C. lasseensis</i>	1	3	3.0 ± 0.42
B. ♀ <i>C. g. tracyi</i> x ♂ <i>C. lasseensis</i>	2	3	2.5 ± 0.57
C. ♀ <i>C. g. gracilis</i> x ♂ <i>C. lasseensis</i>	2	5	1.5 ± 1.21
Subtotal.....	5	11	2.3 ± 0.73
V. F ₁ diploid-hybrids between <i>C. amoena huntiana</i> and <i>C. lasseensis</i> :			
A. ♀ <i>C. am. huntiana</i> x ♂ <i>C. lasseensis</i>	5	23	2.5 ± 0.93
B. ♀ <i>C. lasseensis</i> x ♂ <i>C. am. huntiana</i>	2	2	1.1 ± 0.45
Subtotal.....	7	25	1.8 ± 0.69

A total of 120 crosses between certain taxa that are representative of all three subsections of the section *Primigenia* were carried out in the greenhouse (Table 1, Figure 3). The reason why other taxa in this section were not represented, lies in the fact that the majority of such re-

maining crosses were sporadically made by different investigators and their results as well as mine will be discussed jointly in some detail in a later section of this paper.

Pollen viability was estimated from counts of pollen grains which stained with cotton blue in lacto-

phenol. Only well stained and normal shaped grains were considered viable, and calculations were based on a total of at least 200 pollen grains counted.

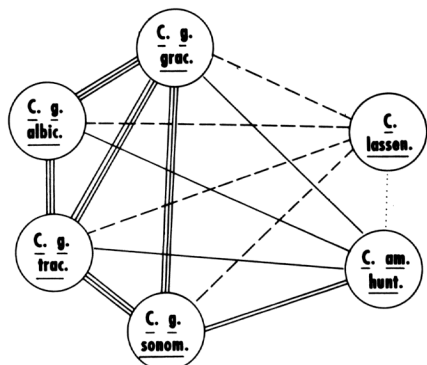


FIGURE 3. Polygon showing inferred crossing relationships from greenhouse progenies.

..... Rarely set seeds; developmental abnormalities in most progenies; few plants reach maturity and are extremely low in fertility.

----- Rarely set seeds; developmental abnormalities in some progenies; most flower buds are low in fertility.

———— Relatively high seed set; normal progeny; almost all reach maturity, but show variable degrees of fertility.

===== Relatively high seed set; normal progeny; progenies are relatively fertile.

===== Abundant seed set; normal progenies with normal fertility.

Buds for cytological study were fixed in 1:3 acetic-alcohol for at least 24 hours, then changed to 70% alcohol and stored in the refrigerator. They were stained in bulk using alcoholic-HCl carmine (Snow, 1963), which gave the most satisfactory results. From these buds slides

were prepared by squashing 2-4 anthers after gentle heating in a drop of 45% acetic acid. Some slides were made permanent using dry ice or liquid nitrogen. After freezing the slide, the cover slip was pried off with a razor blade and the slide was immersed in two changes of absolute alcohol for at least 5 minutes. A drop of euparal and a clean cover slip was placed on the location of the previous cover slip.

RESULTS AND DISCUSSION

In order to better understand the mode of evolution within section *Primigenia*, it becomes necessary to assess the extent of reproductive and developmental differentiation between various species in the section. In addition, observations pertaining to their morphological, eco-geographical, cytological and chemotaxonomical relationships are usually considered in such a study. Thus, data on crosses between different taxa of the section as well as other types of evidence are required. The present study shows the results of interspecific hybridization between some of the species of this section (Table 1, and Figure 3). Of these, hybridization between the diploids *C. amoena* ssp. *huntiana* and *C. lassenensis* proved to be the most difficult due to the presence of a number of barriers to gene exchange between their respective gene pools. These two diploids showed a diversity in their reproductive and developmental patterns, whose magnitude is characteristic of fairly distantly related species or higher taxonomic groupings. By comparison, the phylogenetic relationship between *C. lassenensis* and the tetraploid *C.*

gracilis appeared closer. *C. amoena* ssp. *huntiana* and *C. gracilis* represented the closest relationship of all as witnessed by the ease of hybrid production and their normal pattern of development.

Both prezygotic and postzygotic types of isolation were observed, especially during the formation of the F_1 diploid hybrids. Crosses between *C. amoena* ssp. *huntiana* and *C. lassenensis* proved generally quite unsuccessful. Since most crosses failed to set seeds, it is probable that such failure was due to the inability of the pollen to effect fertilization. In nature, however, the production of these hybrids apparently involved several isolating mechanisms. Acting together, they are extremely efficient in isolating the gene pools of both diploids. The major part of such isolation was due to prezygotic mechanisms, especially spatial, seasonal, and floral isolation.

Spatial isolation of the two diploids, *C. amoena* ssp. *huntiana* and *C. lassenensis* (Figure 2), prevents any sympatric association between them. Such spatial isolation would be reinforced by ecogeographical differences. The single sympatric population discovered in early June, 1966 was located outside the known distributions of both diploids, in the area where both appear closest to each other (small arrow, Figure 2). Should more favorable environmental conditions prevail, more sympatric associations might occur. Notice that species of subsection *Primigenia* are westerly distributed and close to the Pacific coast line, while those that belong to subsection *Flexicaulis* are distributed inland and close to the Sierra Mountains

range (Figure 2). Furthermore, the two diploid subsections are well separated ecogeographically except for this mixed population which represent the only recorded case of sympatric association between any two diploid species of these two subsections.

Seasonal isolation was observed in both greenhouse and wild plants, as *C. lassenensis* was about three or four weeks earlier in starting and ending its flowering season than *C. amoena* ssp. *huntiana*. The sympatric population showed clearly that seasonal variation to a large extent isolated the two diploid gene pools. However, the short period of overlap would be sufficient for cross-pollination. In this population, a relatively large number of pollinating insects such as honey bees, bumble bees, bee flies and several different butterflies, were observed to move from one plant to another. Under such conditions, natural cross pollination could occur, but extensive cytological examination revealed no interspecific hybrids in this mixed colony.

All *Clarkia* species are self-compatible (Lewis, 1953a) and are visited by various kinds of bees, which are undoubtedly the principal pollinators of the genus (Lewis and Lewis, 1955). They all seem to lack heterostyly and various mechanisms of incompatibility between pollen tube and style. However, some of the latter may be operative in interspecific hybridization but this is not known. The flowers of most species are protandrous, and the mature stigma is held above the stamens. Outcrossing is certainly promoted in these species even though they

are self-compatible. But some taxa such as *C. gracilis* ssp. *gracilis* have cleistogamous flowers and are primarily or exclusively self-pollinated. Various intermediate degrees of self-pollination also occur (Lewis, 1953a). Even though every species of *Clarkia* is sympatric with at least one other species over part of its distribution range, no naturally occurring interspecific diploid hybrids have been found (Lewis and Lewis, 1955). Nevertheless, hybridization obviously does occur at least on rare occasions inasmuch as some of the allopolyploid species have been derived from normally self-pollinating species (Lewis and Lewis, 1955).

Morphological observations revealed that *C. lassenensis* was predominantly an inbreeder as the stigma was usually located at the same level or lower than the anthers, whereas *C. amoena* ssp. *huntiana* is predominantly an outbreeder as its stigma is situated higher than the anthers. Such a difference in floral structure might prevent cross-pollination between the two species, or would at least greatly restrict its occurrence. The western populations of *C. lassenensis* have relatively large flowers with a stigma which often is higher than the anthers; it is probably only in this western area where hybridization might occur in nature. All prezygotic mechanisms mentioned above have played a major role in quite effectively isolating the two diploid gene pools.

Postzygotic mechanisms have also played a significant role in the differentiation and diversity observed at present in both taxa. By definition, these mechanisms work after fertilization has taken place and the

hybrid zygotes formed are rendered inviable or produce only weak or sterile hybrids. Postzygotic isolation is usually expressed by three different mechanisms: the inviability or weakness of the F_1 hybrid itself, the complete or partial sterility of vigorous F_1 hybrids, and the production of many weak or sterile F_2 progeny by vigorous fertile F_1 hybrids (Stebbins, 1966). The action of all of them can be characterized in a general fashion as the inability of the parental genes to work together properly in the cells of the hybrid or its progeny or the so-called genic disharmony (Stebbins, 1966). Almost all seed capsules collected from the F_1 diploid hybrid plants contained light-colored chaff which represented aborted ovules or failure of fertilization. A very few had a small number of completely shrivelled or normally appearing seeds, of which some of the latter succeeded in germinating. The completely shriveled brown ones had virtually nothing inside or had ovules which had been fertilized but whose subsequent development was impaired. The partially shriveled brown seeds usually contained a small embryo and/or a small endosperm. Some lacked endosperm tissue.

Hybrid inviability or weakness was observed as a strong isolating mechanism that led to early seedling death. Germination of F_1 hybrid seeds was usually one to two weeks later than normal diploid or tetraploid seeds. F_1 seedlings were also slower in growth than normal ones due to slow differentiation of the hypocotyl and epicotyl. These seedlings showed various degrees of

abnormal development, witnessed by irregular swelling of the epicotyl below the cotyledons. In a few cases, the cotyledons showed abnormal and irregular development and some were fused together with very little or no apical meristematic tissues. In fact, some of them covered or engulfed completely the apex during their irregular differentiation. Some F_1 seedlings that were capable of producing true leaves showed various late developmental abnormalities affecting the leaves and stems.

Developmental or genic hybrid sterility which is usually rare in plants (Stebbins, 1966) was observed in a few of those F_1 seedlings that succeeded in growing beyond the above mentioned phases. They showed irregularities during their floral differentiation. Generally, all F_1 diploid hybrids were quite sterile due to abnormal development of male and female gametophytes and/or meiotic breakdown. In many cases, they bore relatively small sized and abortive flowers, of which some developed all or in part abnormally. The irregularities observed included flowers which had more than or less than the normal four petals, eight anthers, and four-lobed stigma. Certain cases provided evidence for reversion of development of at least one anther into an extra petal. These floral irregularities were in low frequency, most flowers showing normal development, but all flowers were of extremely low fertility.

Segregational hybrid sterility is caused by abnormal segregation at first anaphase of whole chromosomes or chromosome segments. The unbalanced gametes resulting are usually inviable and probably represent the

principal factor of the extremely low fertility observed in F_1 diploid hybrids. Most M_1 plates of these hybrids had 14 unpaired chromosomes. Upon doubling the chromosome number of plants with normal flower development, normal chromosome segregation and fertility were restored.

F_2 hybrid breakdown was observed in the progeny of selfed F_1 plants. Another group of plants that showed what might be termed F_2 breakdown was the hybrid progeny of crosses between F_1 diploid hybrids and natural allotetraploids. The majority of these crosses had a better seed setting than their F_1 parents. Most seeds germinated to produce healthy and vigorous plants. However, all of them except two, were quite sterile and did not set seeds upon selfing or further crossing. Probably the main factor causing their infertility was irregular meiotic chromosome orientation and segregation, since most of their PMCs showed a majority of univalent formation on M_1 plates.

It is obvious that the two diploid taxa *C. amoena* ssp. *huntiana* and *C. lassenensis* representing subsections *Primigenia* and *Flexicaulis*, are separated by a multitude of strong isolating mechanisms. Such well developed genetic barriers have probably set the stage for a relatively high ratio of allotetraploids which comprise 1/3 of the species in this genus (Lewis 1953a; Lewis and Lewis, 1955). F_1 hybrids between these two diploids manifested different barriers to gene exchange in a variety of developmental abnormalities that could be classified into those affecting vegetative structures as opposed to those affecting repro-

ductive structures. In general, the majority of postzygotic isolating mechanisms are probably the end product of either disharmonious gene action between parental genomes in the hybrid and/or abnormal segregation of parental chromosomes.

Developmental abnormalities and reproductive isolation were at maximum between the two diploids and at minimum between *C. amoena* ssp. *huntiana* and the tetraploid *C. gracilis* ssp. *sonomensis* (see Figures 3, 4). The present data suggesting

enlarging the gene pool of each and probably serving to retain close homology between their genomes (Abdel-Hameed, 1967). Evidence has accumulated recently which indicates a much higher frequency of natural hybridization and intergenome connections on the same ploidy-level (Zohary and Feldman, 1962) or on different ploidy-levels (Anderson, 1953) than was earlier realized.

Interestingly, *C. amoena* which is considered the oldest and most primitive member of the genus (Lewis and Lewis, 1955), when crossed with *C. arcuata* (the other member of subsection *Flexicaulis*, see Figure 1) yields few diploid hybrids. These produced very small plants that gave no flower buds with meiotic stages (Hakansson, 1946). Thus, inferring an even greater genetic isolation than that observed between *C. las-senensis* and *C. amoena*. In other words, the two diploid subsections, *Primigenia* and *Flexicaulis* are definitely well separated by strong barriers to gene exchange. Within the same subsection, genetic isolation between species seems much weaker than that observed between those of different subsections as witnessed by the relative ease of producing F_1 diploid hybrids between any two species in subsection *Primigenia* i.e., *C. amoena*, *C. rubicunda*, and *C. franciscana* (Lewis and Raven, 1958). Furthermore, Lewis and Raven (1958) concluded that *C. franciscana* is chromosomally more similar to *C. rubicunda* than to *C. amoena*, and *C. rubicunda* is probably more similar to *C. amoena* than to *C. franciscana*. They also concluded that *C. rubicunda* is probably the parent of *C. franciscana* while

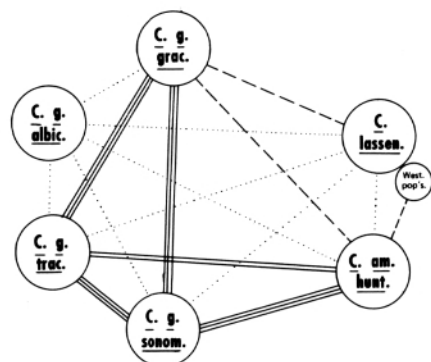


FIGURE 4. Polygon showing inferred relationships from sympatric and allopatric associations in nature.

- No gene flow.
- Very little gene flow.
- ===== Some gene flow occurs.
- ===== Possibility for considerable gene flow.

a closer phylogenetic relationship between the latter two taxa is also supported by frequent sympatry between them in certain mixed populations where it is difficult to differentiate the two morphologically. Such observations may indicate a continuous gene flow between the two, hence,

the much older *C. amoena* is perhaps ancestral to both. Such parent to offspring phylogenetic relationship is apparently the case throughout the genus (Lewis, 1962).

The fact that *C. amoena* and *C. lassenensis*, which are presumably fairly closely related, show a magnitude of isolating mechanisms commonly observed in crosses of different genera or even higher taxonomic groupings, is not surprising because of the genetic diversity of the genus *Clarkia*. Lewis and Lewis (1955) lucidly illustrated that barriers to gene exchange between many *Clarkia* species are extremely well developed. They made several thousand interspecific pollinations involving at least 202 species combinations. Of these only 55 combinations produced viable hybrids. Additional crosses have been made by several other workers. The vast majority of interspecific hybrids that were produced showed a great reduction in fertility. The basis of this low fertility in interspecific hybrids in *Clarkia* lies primarily in the extensive structural rearrangement of the chromosomes of most species resulting in highly irregular meiotic behavior of the hybrids (Lewis 1953a; Lewis and Lewis, 1955; Lewis and Raven, 1958). The highly irregular meiotic behavior and the lack of homology between parental chromosomes observed in F_1 diploid hybrids between *C. amoena* ssp. *huntiana* and *C. lassenensis* (Abdel-Hameed, 1967), indicate a karyotype diversity between relatively two close species that could happen only if we assume extensive rearrangement within their respective genomes. Such observations have led Lewis

and Raven (1958) to conclude that the accumulation of structural rearrangements, primarily reciprocal translocations, has undoubtedly played a major role in the formation of barriers to gene exchange and hence to speciation on the diploid level in *Clarkia*. At the same time, such well developed isolating mechanisms have set the stage for instantaneous speciation on the polyploid level through allopolyploidy. In other words, the principal consequences of interspecific hybridization in such a group of species would seem to be the production of a number of allopolyploid species (Lewis, 1953a). This is evident in section *Primigenia* since morphological, ecological, cytological and chromatographic comparisons illustrate the fact that the allotetraploid *C. gracilis* does indeed combine features and genomes of the two diploid subsections (Abdel-Hameed, 1967).

The appearance of strong and effective genetic isolation, especially so, on the diploid level between otherwise fairly closely related species, is also correlated with a characteristic mode of speciation. The mode of speciation in the section *Primigenia* has been apparently dependent on two major factors, i.e., rapid speciation and catastrophic selection.

Rapid speciation (as defined by Lewis, 1953a) refers to the process whereby certain natural populations of *Clarkia* differentiate reproductively from each other within a few generations to a point where they become genetically isolated. These genetically isolated populations are probably short lived, but a few may become successful species. Such a characteristic pattern of evolution

in *Clarkia* is not only rapid but seems to occur with great frequency (Lewis, 1953a). Lewis (1953a) argued that while gradual segregation of geographic races also occurs, the success of the genus seems not to lie so much in the gradual segregation of adaptive races as in the production of a multiplicity of locally adapted, genetically isolated and often ephemeral segregates. The colonial habit probably has been a factor in rapid differentiation as a result of random fixation due to reduction of population size and inbreeding, particularly in the initial stages of colony formation. Five years later, Lewis and Raven (1958) presented more evidence to support Lewis's original hypothesis of rapid evolution in *Clarkia*. They proposed that *C. franciscana* had its origin *in situ* from *C. rubicunda* (both are members of subsection *Primigenia*, see Figure 1) as a consequence of rapid reorganization of the chromosomes due to a temporary presence of a mutator genotype conducive to extensive chromosome breakage. Interestingly, *C. franciscana* is known from a single population in nature (in the Presidio at San Francisco, California — see large curved arrow in Figure 2). A similar mode of origin by such a rapid chromosomal reorganization was suggested in other species of *Clarkia*, i.e., *C. lingulata* from *C. biloba* (Lewis and Roberts, 1956) and *C. exilis* from *C. unguiculata* (Vasek, 1958). In all these examples, the derivative populations grow adjacent to the parental species, which they resemble closely in morphology, but from which they are reproductively isolated because of multiple structural

differences in their chromosomes. The spatial relationship in each parental species and its derivative suggests that differentiation has been recent. The repeated occurrence of the same pattern of differentiation in *Clarkia* has led Lewis and Raven (1958) to suggest that this pattern of rapid reorganization of chromosomes has been an important mode of evolution in the genus.

Catastrophic selection is a process whereby an entire population is suddenly eliminated by an environmental extreme or stress, except for one or a few individuals which are exceptionally well adapted to the new environment and able to pass through the bottleneck. Such a pattern of selection would provide the conditions necessary for the establishment of a population characterized by deviant genomes. By elimination of the parental population, catastrophic selection would isolate the survivors and their progenies in an open habitat to which they are adapted. Favorable alleles improving fertility would accumulate and provide more stable and balanced genomes genetically isolated from the parental population. Lewis (1962) provided evidence for catastrophic selection in *Clarkia* based on the observation that ecologically marginal populations in several species have suddenly become extinct. Extinction in all cases resulted from exceptional drought, which throughout the history of the genus has been the most likely cause of catastrophic selection associated with speciation. He concluded also that species pairs in *Clarkia* are related as parent to offspring rather than as siblings.

SUMMARY AND CONCLUSION

In section *Primigenia*, diploids of different subsections are well differentiated reproductively and developmentally as witnessed by a multitude of strong pre- and postzygotic isolating mechanisms. Whereas diploids of the same subsection are not so well isolated genetically. Such well developed genetic isolation between the gene pools of subsections *Primigenia* and *Flexicaulis* has apparently set the stage for polyploidy. The fact that the third subsection, i.e., *Jugales*, consists of a single allotetraploid that combines morphological, ecogeographical, cytological and chemotaxonomical features of both diploid subsections, is quite interesting. It does not only sustain the idea that polyploidy is the by-product of such well developed isolating mechanisms, but also that polyploidy seems to knit the six species designated in Figure 1 into one group of fairly close relatives that we recognize as section *Primigenia*. On the other hand, the appearance of genetic isolation whose magnitude is common only between different genera or families to otherwise fairly close relatives like *C. amoena* ssp. *huntiana* and *C. lassenensis* is apparently correlated with a characteristic mode of speciation for this group.

The classical mode of evolution through the slow and stepwise accumulation of point mutations resulting in race formation and ultimately in genetic and/or geographical isolation is indeed involved. The recent accumulated evidence (Lewis, 1953a, Lewis and Raven, 1958) strongly indicate that speciation in section *Primigenia* and in *Clarkia*

in general is for the most part a rapid process. Such a rapid process, i.e., occurring within few generations is achieved on the same ploidy level through extensive and short term chromosomal reorganization or through catastrophic selection. It may also result in a change of the ploidy level as witnessed in the instantaneous speciation of the allotetraploids that constitute 1/3 of the species in the genus. In both cases the process of speciation occurs *in situ* and the resulting species are related as offspring rather than as siblings.

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